

	TCACAAGAACATCGAACCATGTAGAGAG	-855
<b>AP-1 site</b>	<b>CCAAT box</b>	
<b>ACTTAGTTGCTTTAACAGAATTGGGCACGGGCTGTTCA</b>	<b>GAAACAAACAAATCTTCACAT</b>	-795
CCATTATAATGATAGCATTAGTGTAGTTAGCAAATGTTACTG	<b>TGAGCCTGTTAT</b>	-735
GTGCTGAGCCTGCTATGTAAGAAGTGTGCTCTCTGGACAGGAGACAGAA	<b>TACTAAACAA</b>	-675
CACAACTACTGATCTTGCTGCCATGCTCCTCACTTCATATGGTATCAGCAATT		-615
TAGCACCAACAAACGTCCTTAGAGAACCCAGCCTTCTCATTCTGGTTCTAGTGGCTTG		-555
AGTAGACTGACCCCAGCTACCCAAAGTGGATTGACTCTAGAACATTCAATTCTAGC		-495
<b>CCAAT box</b>	<b>CCAATAAAATGTCAAGTACAGGACTTTATTGAAAGCATTCA</b>	-435
ACACTAAACATTTGTAACAAATA	<b>AGGATGTTAGAAATTCTCTAGAAAGGAAGCTATGA</b>	-375
<b>TATAAA</b> TGGTTGCTAGATGGGTCTAGTAGATGGTGGCC	<b>TGCTTGTACTGCCTTGT</b>	-315
GTATTGTGCTACCATAAGCCCTCCCCAAACTGTA	<b>CTCTGGCTCTGGCATTTCCGCTCTTT</b>	-255
CAACCAGATGGTCAGCTCTAAGTGAAGGAGACACATCTCAACATGCTTGGTTCTAGC		-195
ACAACAGAACAGGCTCAAACACATACTGCTAAAGAAACATACCTGTAGGGATTAGCAGCA		-135
<b>Inverted CCAAT Box/E2F-like site</b>		
<b>TGGCCATGAGGCATTGGCGTTCTATCA</b>	<b>TGGGAACTCAGGTTCTGGTGCTCCAGTAC</b>	-75
<b>TCTACTGGCTGATACCACATCCTACAA</b>	<b>TTCACTTCATAGGCTTGGGTTCCCTGCTCTGGC</b>	-15
<b>+1 AP-1 AP-4</b>		
<b>TGAATAGGTGGTCCACTCTGAGTCATCAGCTGTGGGTGATGATGTGGTCACTGCATGATT</b>		46
<b>CRE</b>	<b>Ikaros</b>	
CTCACACAAGCACCCAGAGGACGT	<b>CATCAGGCAGAGGGCAGTGGGGTGGGCAGCATTTAC</b>	106
<b>Start of GrB-NIC cDNA</b>		
<b>CBF/AP-1</b>	<b>*</b>	<b>CRE</b>
<b>AGAAAATCTGTGATGAGACACCACAAAACCAGAGGGAACATGAAGTC</b>	<b>ACTGAGCCTGCT</b>	166
	<b>M K S L S L L</b>	<b>7</b>
<b>(GrB)TATA box</b>	<b>NF-AT site</b>	
<b>CCACCTTTCCCTCCCAAGAGCTAAAGAGAGCAAGGAGGAACAAACAGCAGCTCCAA</b>		226
<b>H L F P L P R A K R E Q G G N N S S S N</b>		<b>27</b>
<b>Start of human CTL GrB cDNA</b>		
<b>CCAGGGCAGCCTTCCCTGAGAACATGCAACCAATCCTGCTCTGCTGGCCTTCCCTGCT</b>		286
<b>Q G S L P E K M Q P I L L L A F L L L</b>		<b>47</b>
<b>GCCCCAGGGCAGATGCAGGGGAGATCATGGGGGACATGAGGCCAAGGCCCCACTCCGCC</b>		346
<b>P R A D A G E I I G G H E A K P H S R P</b>		<b>67</b>
<b>CTACATGGCTTATCTTATGATCTGGATCAGAAGTCTCTGAAGAGGTGCGGTGGCTTCCT</b>		406
<b>Y M A Y L M I W D Q K S L K R C G G F L</b>		<b>87</b>
<b>GATACAAGACGACTTCGTCGTCAGCAGCTGCTCACTGTTGGGGAGCTCCATAAATGTCAC</b>		466
<b>I Q D D F V L T A A H C W G S S I N V T</b>		<b>107</b>
<b>CTTGGGGGCCACAATATCAAAGAACAGGAGGCCACCCAGCAGTTATCCCTGTGAAAAG</b>		526
<b>L G A H N I K E Q E P T Q Q F I P V K R</b>		<b>127</b>
<b>ACCCATCCCCATCCAGCCATAATCCTAAGAACCTCTCCACGACATCATGCTACTGCA</b>		586
<b>P I P H P A Y N P K N F S N D I M L L Q</b>		<b>147</b>
<b>GCTGGAGAGAAAGGCCAAGCGGACAGAGCTGTGCGAGCCCTCAGGCTACCTAGAACAA</b>		646
<b>L E R K A K R T R A V Q P L R L P S N K</b>		<b>167</b>
<b>GGCCCAGGTGAAGCCAGGGCAGACATGCAGTGTGGCCGGCTGGGGCAGACGGCCCCCT</b>		706
<b>A Q V K P G Q T C S V A G W G Q T A P L</b>		<b>187</b>
<b>GGGAAACACTCACACACTACAAGAGGTGAAGATGACAGTCAGGAAGATCGAAAGTG</b>		766
<b>G K H S H T L Q E V K M T V Q E D R K C</b>		<b>207</b>
<b>CGAATCTGACTTACGCCATTATTACGACAGTACCTGAGTTGTGCGTGCGGTGGGGACCCAGA</b>		826
<b>E S D L R H Y D S T I E L C V G D P E</b>		<b>227</b>
<b>GATTAAGGAGACTTCCTTAAAGGGGGACTCTGGAGGCCCTTGTGTAACAAAGGTGGC</b>		886
<b>I K K T S F K G D S G G P L V C N K V A</b>		<b>247</b>
<b>CCAGGGCATTGTCTCTATGGACAAACATGGCATGCCCTCACGAGCCTGCACCAAAGT</b>		946
<b>Q G I V S Y G R N N G M P P R A C T K V</b>		<b>267</b>
<b>CTCAAGCTTGTACACTGGATAAAAGAACCATGAAACGCTACTAACTACAGGAAGCAAA</b>		1006
<b>S S F V H W I K K T M K R Y *</b>		<b>281</b>
<b>CTAAGCCCCCGCTGTAATGAAACACCTCTGGAGCCAAGTCCAGATTACACTGGGAG</b>		1066
<b>AGGTGCCAGCAACTGATAAAATACCT</b>		1092

Figure 1

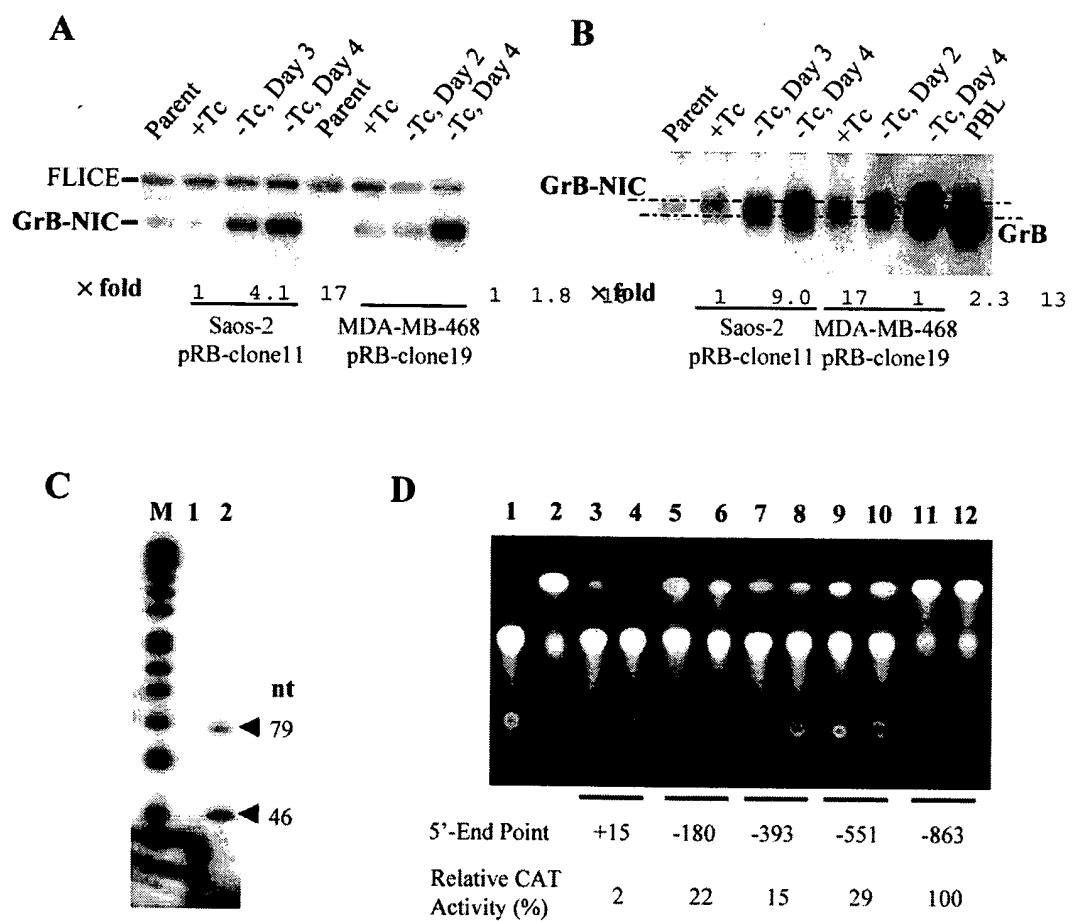


Figure 2

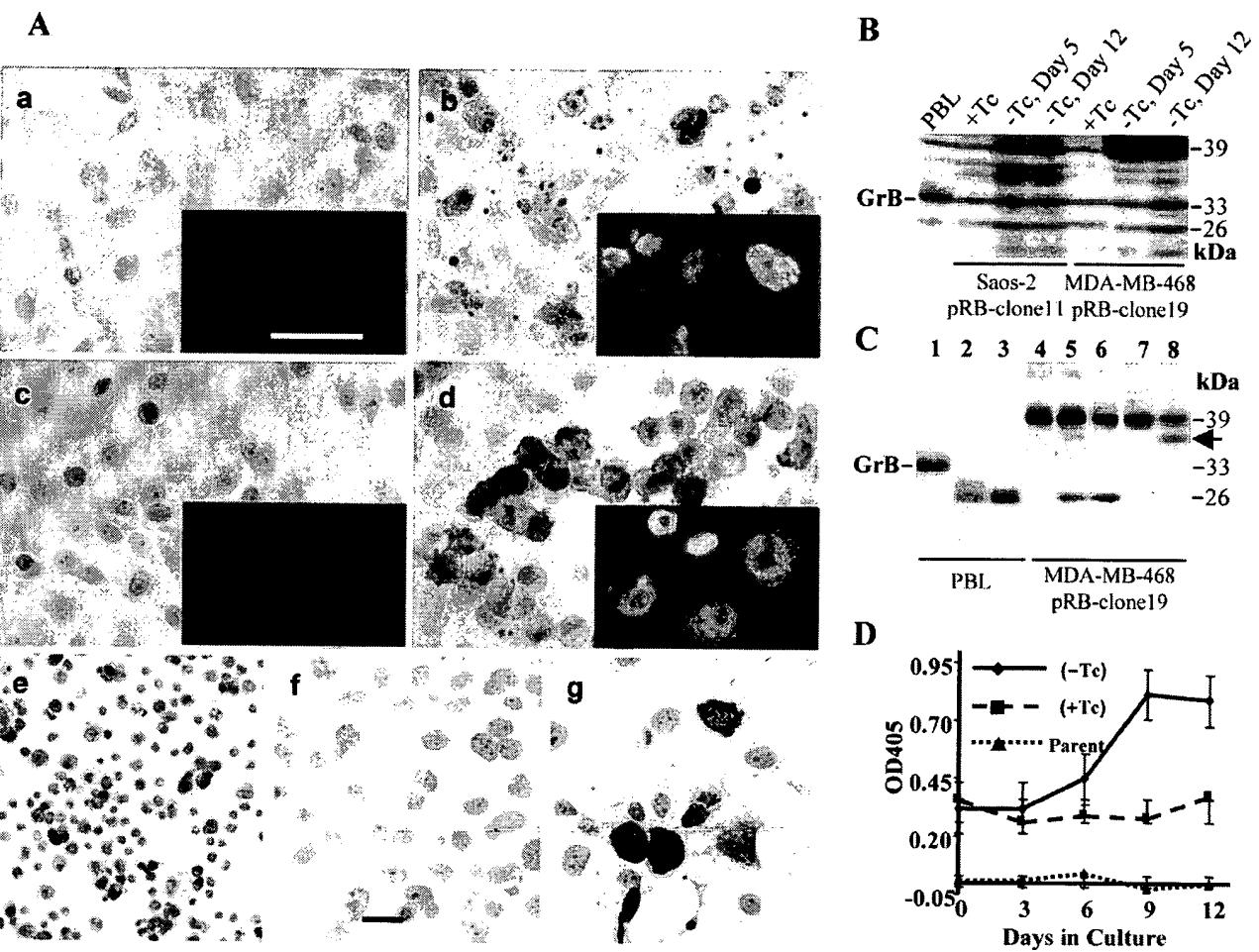
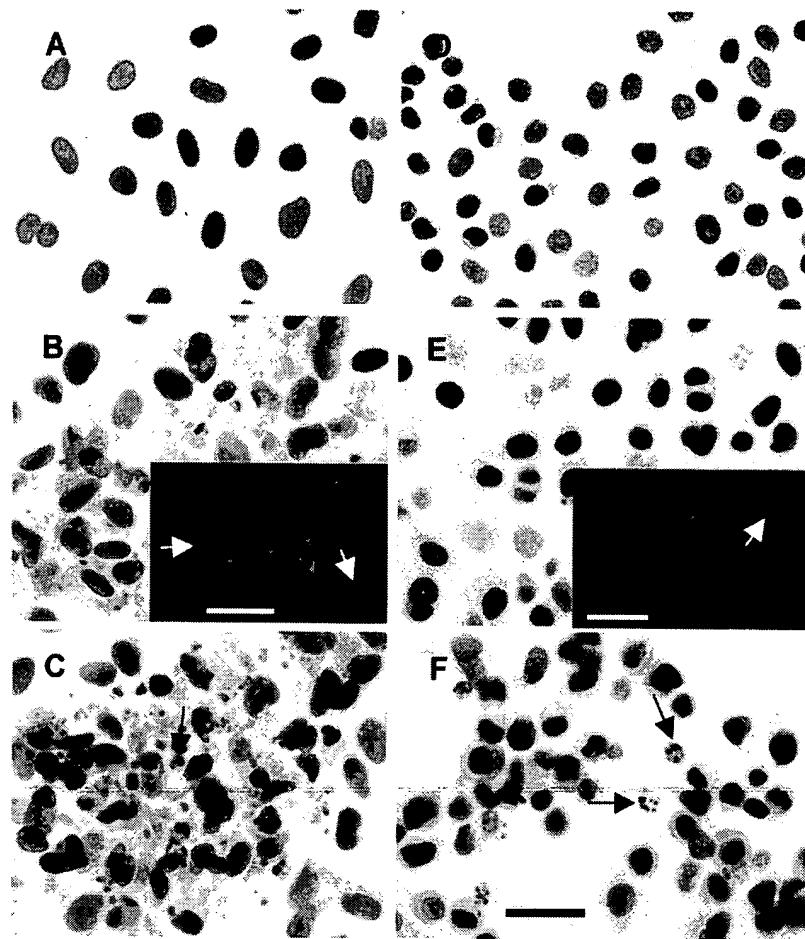


Figure 3

Saos-2 pRB-clone 11    MDA-MB-468 pRB-clone 19



Saos-2 pRB-clone 11

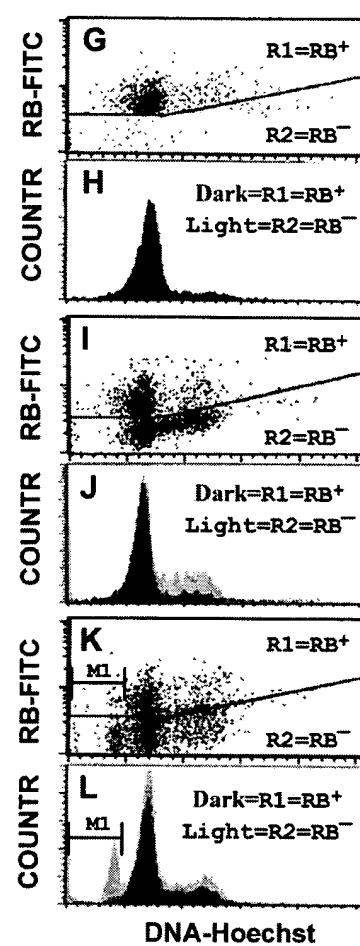


Figure 4

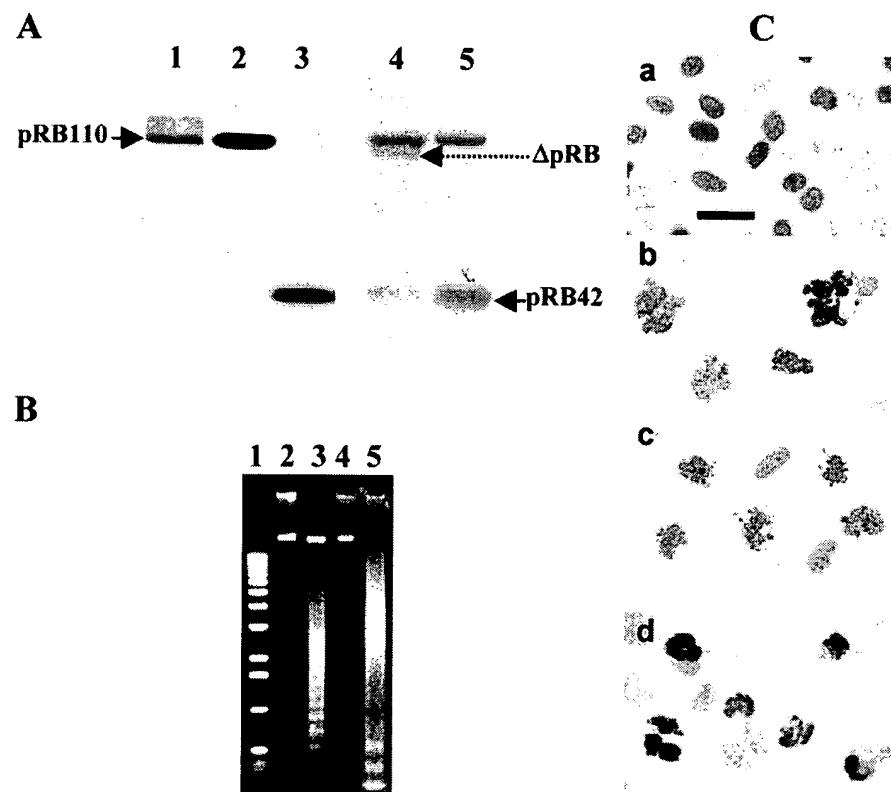


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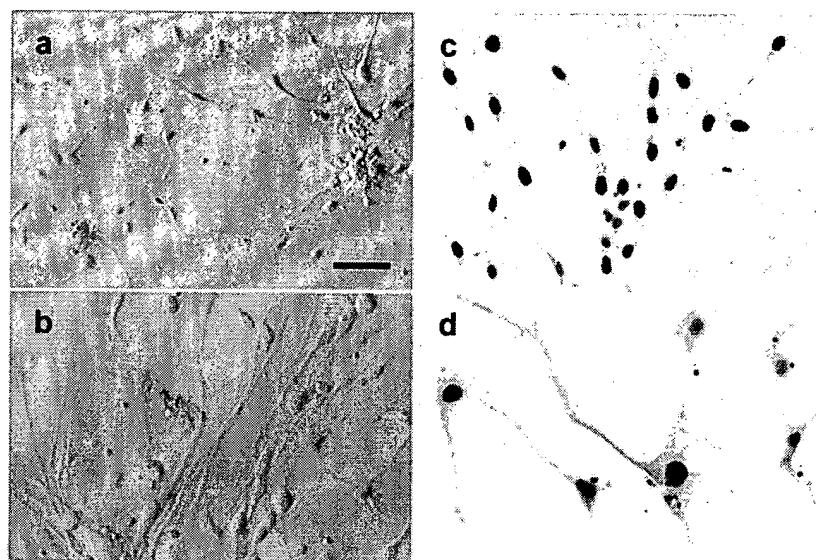


Figure 6

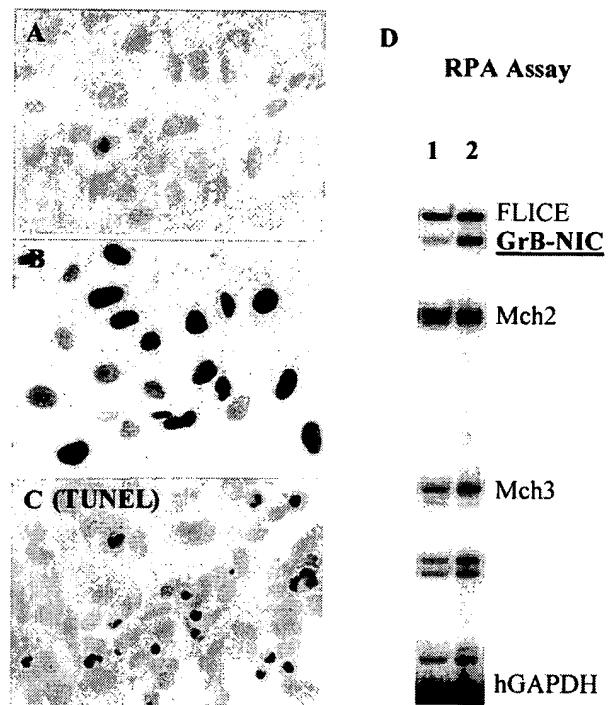
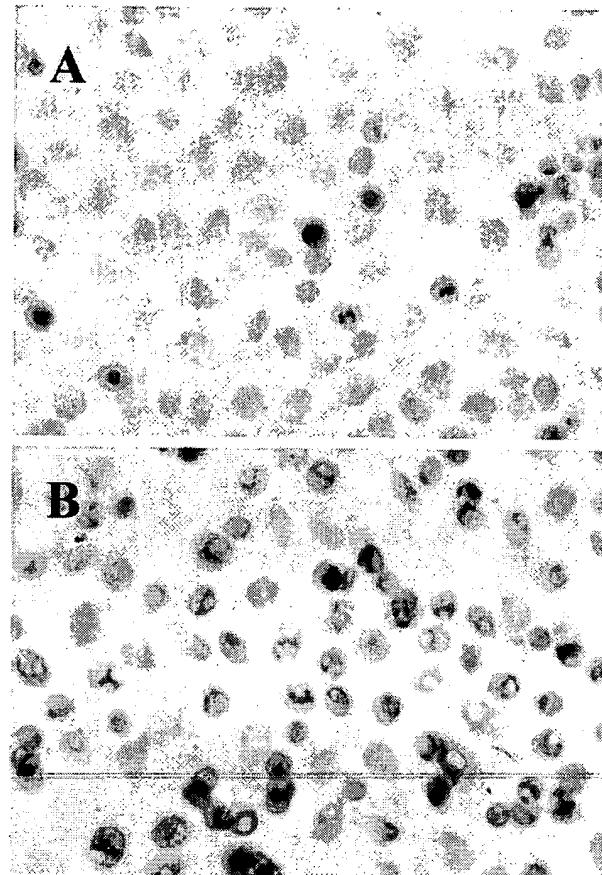


Figure 7

**MDA-MB-468 pRB-clone 19**



**Figure 8**

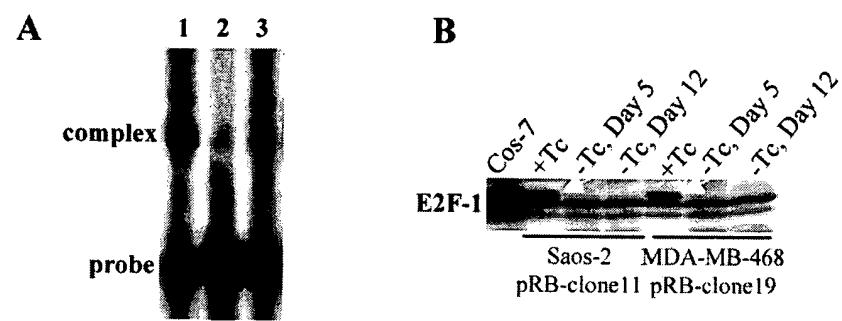


Figure 9

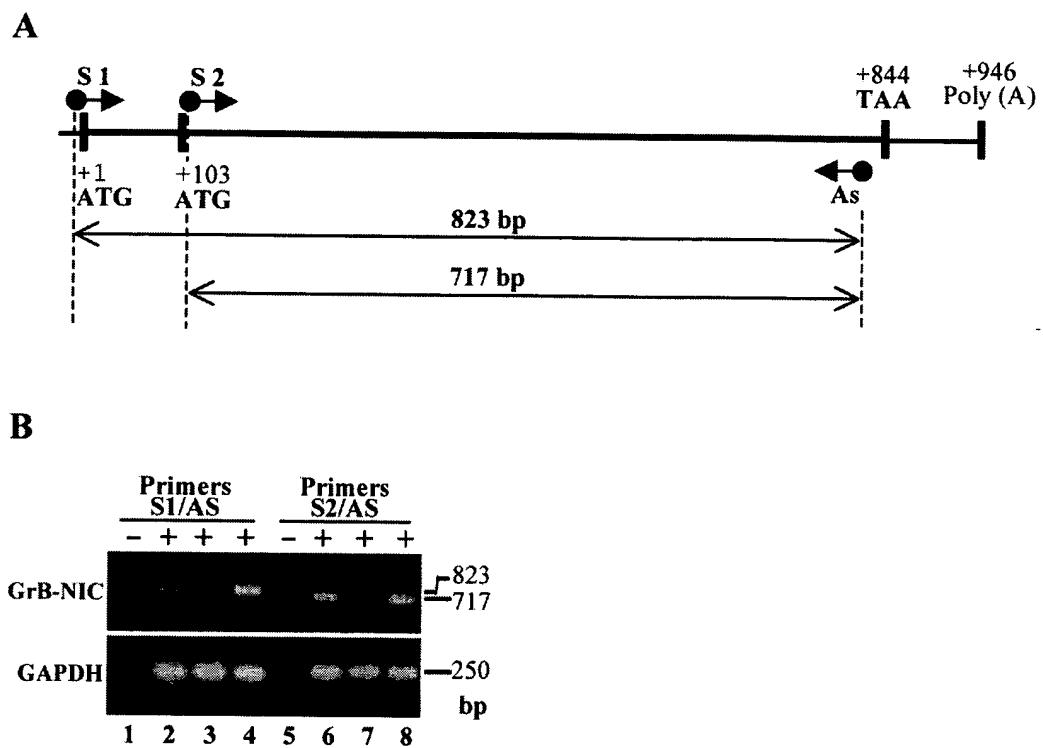


Figure 10

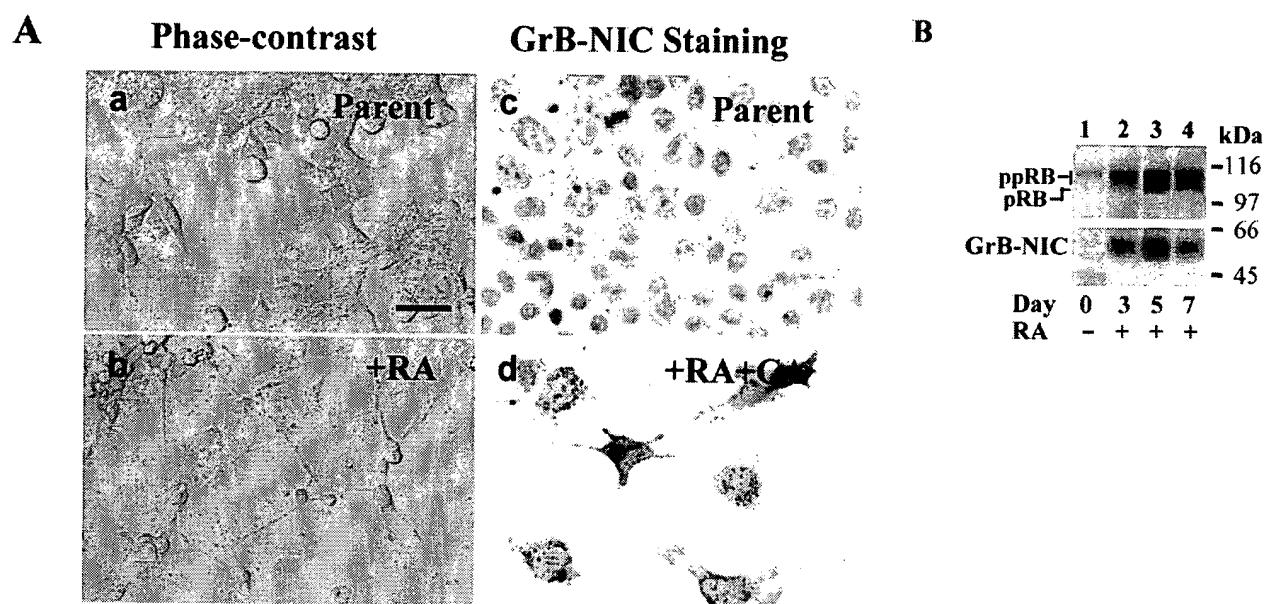


Figure 11

### Breeding Scheme:

The RB and GrB-NIC double-mutant mouse embryos extended survival to approximately embryonic day 19.5

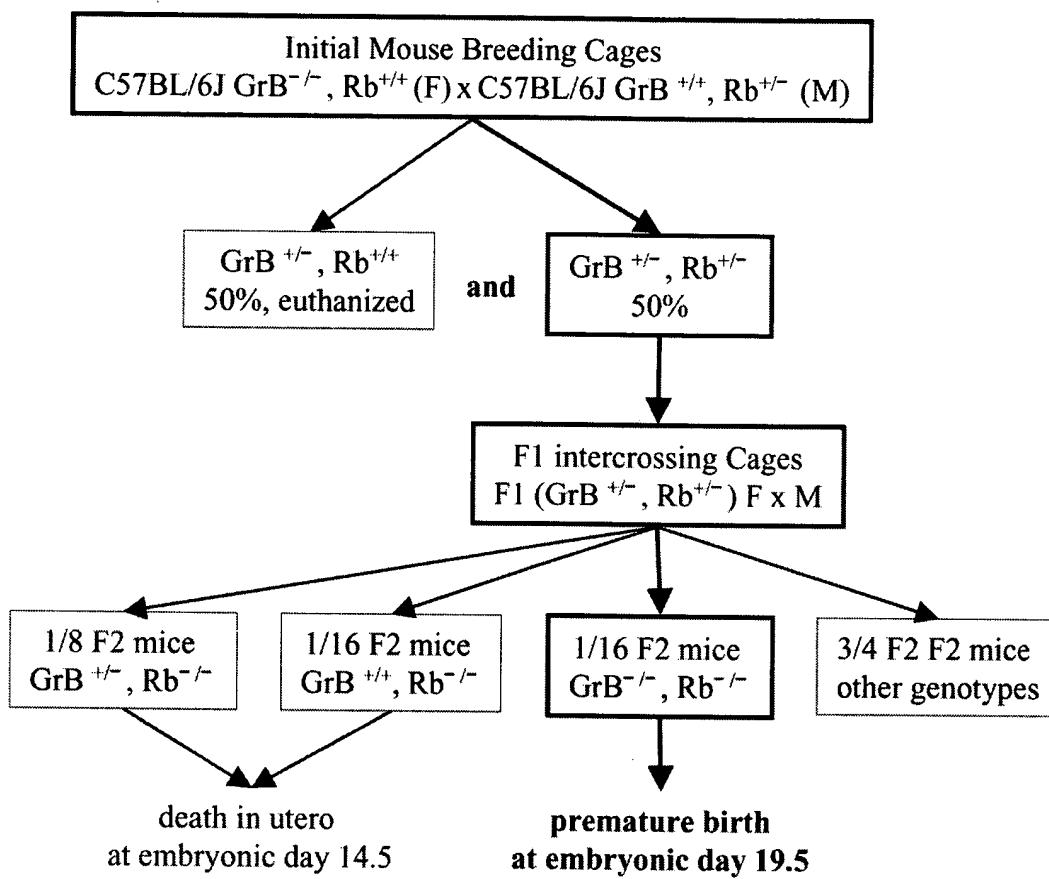


Figure 12

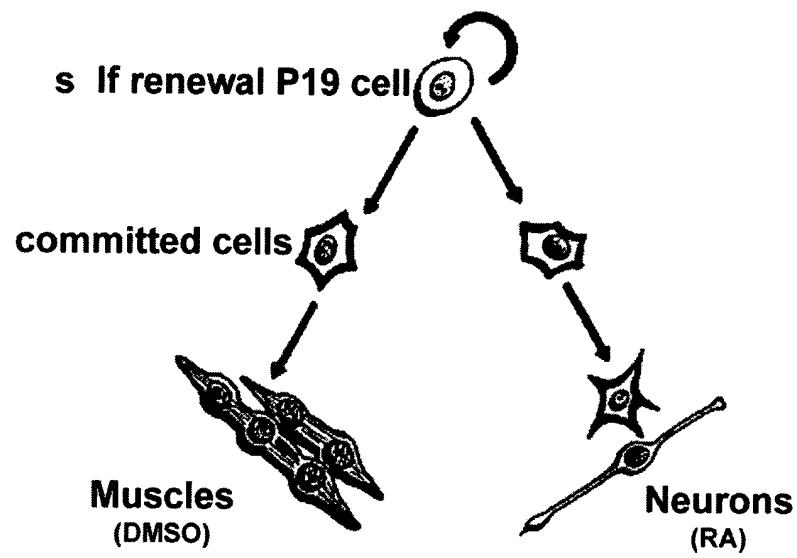
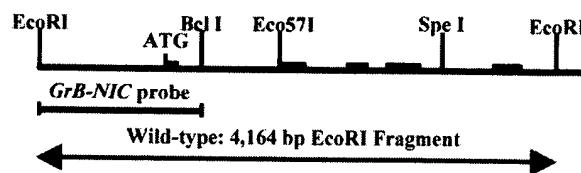
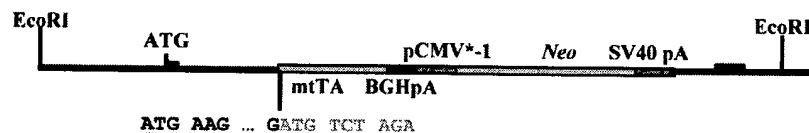


Figure 13

### Genomic Map of the Murine GrB-NIC (GrB) Gene



### Structure of the Targeting Vector



### Schematics of the Disrupted GrB-NIC (GrB) Allele

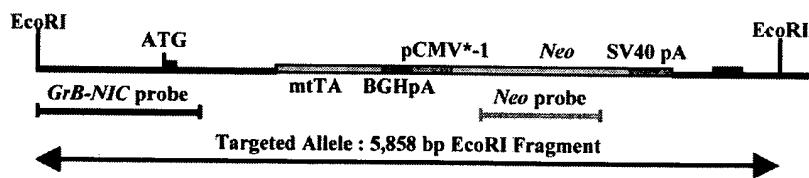


Figure 14

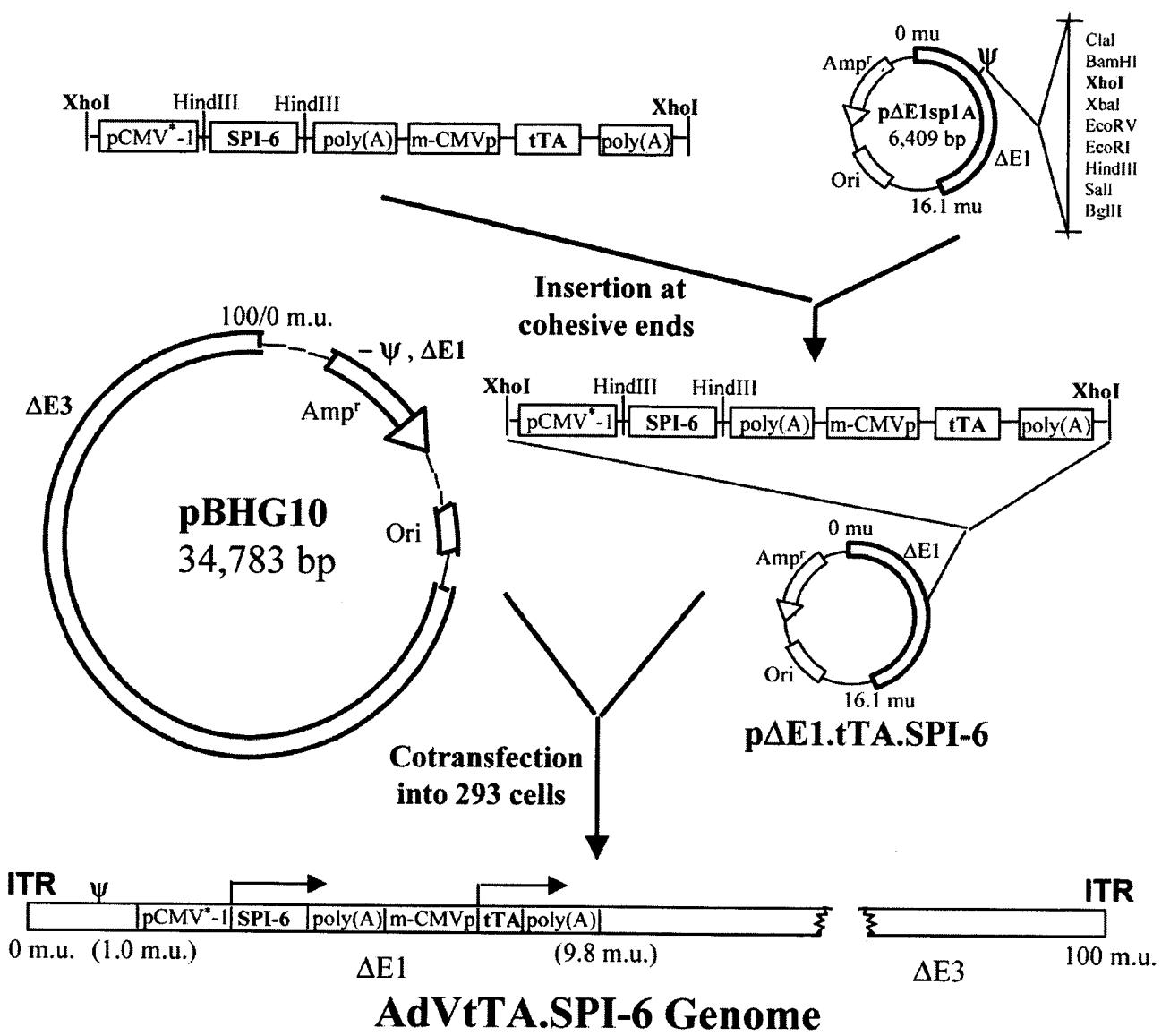


Figure 15

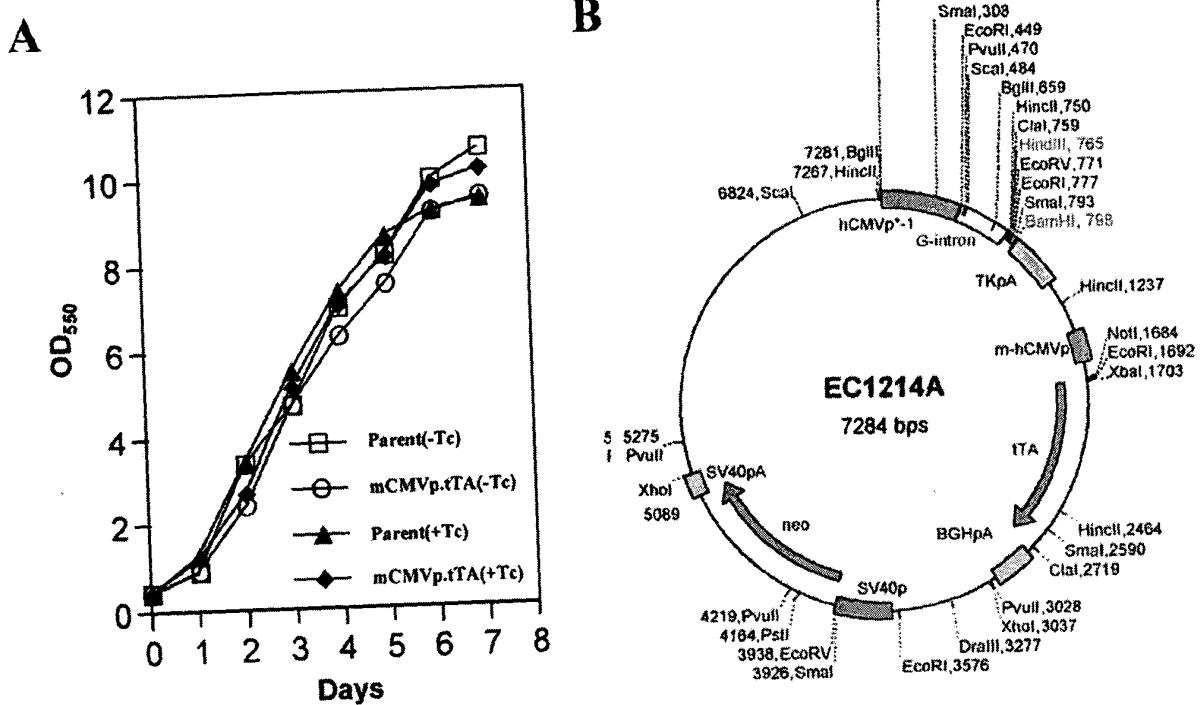


Figure 16

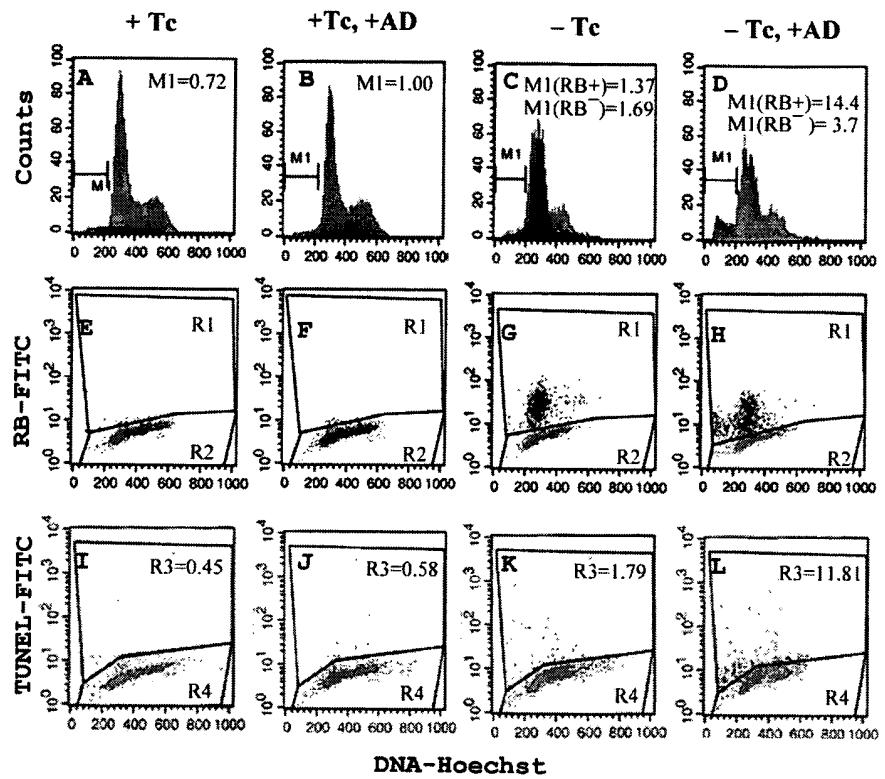


Figure 17

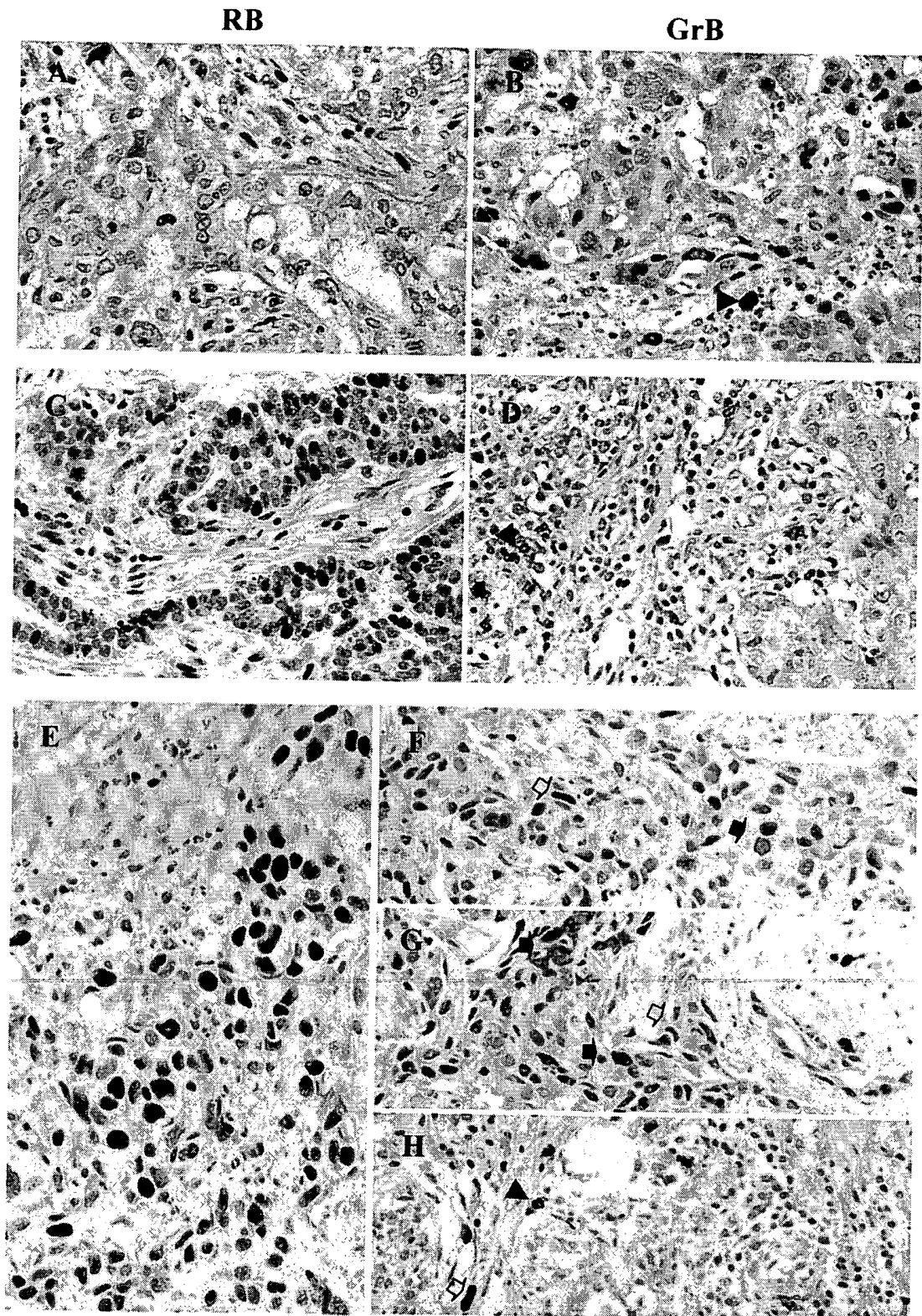


Figure 18

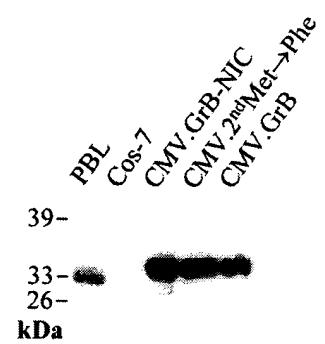
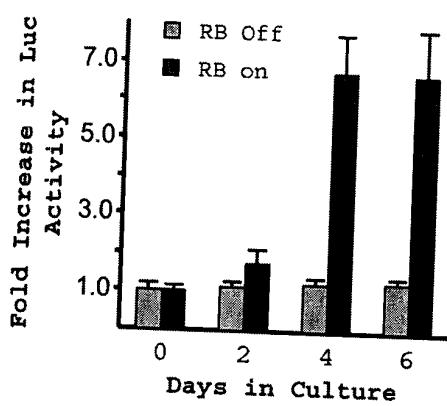


Figure 19

**A**

Saos-2.pRB clone with chromosomally integrated pGrB-NIC-Luc



**B**

MC3T3 Stably Transfected with pGrB-NIC-Luc

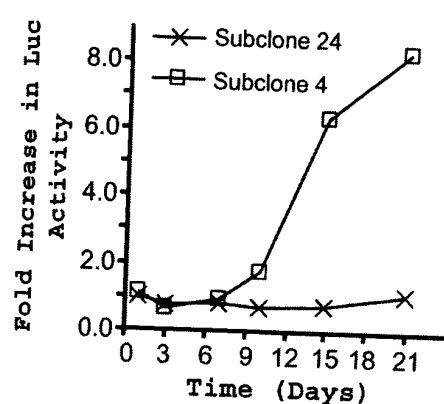


Figure 20